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Relationship of serum antioxidant micronutrients and sociodemographic factors to cervical neoplasia: a case-control study

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Abstract

Background: Although there have been some epidemiological studies on the effects of diet and nutritional status on cervical carcinogenesis, evidence for a protective effect of antioxidant micronutrients against cervical neoplasia is insufficient. The relationship between serum antioxidant micronutrients and sociodemographic factors and the risk of cervical neoplasia was investigated in this multi-center, case-control study.
Methods: The study population included women with histopathological diagnosis of cervical intraepithelial neoplasia (CIN) 1 (n=147), CIN 2/3 (n=177), cervical cancer (n=160), and a control group (n=378). Epidemiological data were collected and the serum concentrations of β-carotene, lycopene, zeaxanthin plus lutein, retinol, α-tocopherol, and γ-tocopherol were measured using reverse-phase, gradient high-pressure liquid chromatography.
Results: Cervical cancer was found to be associated with older age, increased body mass index, and lower socioeconomic status as measured by education level and income. The mean serum concentrations of β-carotene, lycopene, zeaxanthin plus lutein, retinol, α-tocopherol, and γ-tocopherol of cervical cancer patients were significantly lower than those of control subjects. Odds ratio adjusted for age, smoking status, alcohol consumption, and human papillomavirus infection status revealed a significant gradient of decreasing risk of CIN 1, CIN 2/3, and cervical cancer with increasing serum concentrations of most antioxidant micronutrients.
Conclusions: The results of this study show an inverse association between serum antioxidant micronutrient concentrations and the risk of cervical neoplasia. These results suggest that antioxidant micronutrients play a role in the prevention of cervical carcinogenesis.
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Keywords: antioxidant; cervical cancer; cervical neoplasia; micronutrients; sociodemographic factors.

Introduction

Cervical cancer is the second most common cause of cancer deaths in women worldwide, with ~510,000 new cases and 288,000 deaths occurring globally each year (1). Research from the last decade has shown definitively that human papillomavirus (HPV) is the primary causal agent in cervical carcinogenesis (2). The recognition of the central role that high risk HPV plays in the etiology of cervical cancer has led to the development of a prophylactic vaccination as a new means of cervical cancer prevention. However, the impact of this strategy on cervical cancer prevention is unclear at present. HPV vaccines will reduce, but not eliminate, the risk of cervical cancer. Screening programs will continue to be important interventions for cervical cancer, although the procedures used for screening may need to be adapted (3).

Cervical cancer associated with HPV infection results from a continuous process, starting with a normal cervical epithelium, progressing to intraepithelial lesions, and finally, invasive cervical cancer. Although oncogenic HPV infections have been established as a cause of cervical cancer, most HPV infections are transient and rarely progress to significant cervical lesions (4). Epidemiological evidences suggest that

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many HPV cofactors, including antioxidant micronutrients, may alter the natural history of HPV infection and modulate the progression of HPV infection to cervical intraepithelial neoplasia (CIN) and invasive cancer. These cofactors appear to be directly related to the physiological and immunological state of the cervix (5).

Nutritional status may be an important cofactor affecting both persistent HPV infection and progression to cervical neoplasia (6). Over the past few decades, a large body of epidemiological evidence indicates that higher consumption of dietary antioxidants and increased serum concentrations of antioxidant micronutrients may be associated with a reduced risk of several cancers, including cervical cancer (7). Antioxidant micronutrients are important in protecting against oxidative stress, which may have a role in cancer and cardiovascular diseases (8). Current research is focused on identifying the association of serum antioxidant micronutrients (β-carotene, lycopene, zeaxanthin plus lutein, retinol, α-tocopherol, and γ-tocopherol) and sociodemographic factors with the risk of CIN and cervical cancer.

Materials and methods

Subjects recruitment

The data were obtained from a case-control study of cervical cancer conducted between June 2006 and July 2007 at six academic medical centers in Korea: Yonsei University Gangnam Severance Hospital, National Cancer Center, Korea University Guro Hospital, Chungnam National University Hospital, Gachon University Gil Medical Center, Kyunghee University East-West Neo Medical Center, Ajou University Medical Center, and The Catholic University of Korea Holy Family Hospital. The participants for this study were recruited from women undergoing Papanicolaou (Pap) smear testing and the Hybrid Capture® 2 test for screening of cervical cancer. Eligibility criteria were applied to both cases and controls and included not being pregnant at the time of recruitment, having no history of cancer, and having an intact cervix. After an initial Pap smear, patients with abnormal test results were evaluated with colposcopy. Case subjects comprised those patients who had an abnormal Pap smear result, colposcopy-directed biopsy, and a histopathological diagnosis of CIN 1, CIN 2/3, or invasive cervical cancer. All histological diagnoses were reviewed by two pathologists. There were 147 CIN 1, 177 CIN 2/3, and 160 cases of invasive cervical cancer in the case group. A total of 378 control subjects consisted of healthy women who had no history of an abnormal Pap smear and a normal Pap smear on the day of recruitment. Less than 5% of cases and controls offered the chance to participate refused to be interviewed. All patient specimens were collected and archived using protocols approved by the Institutional Review Boards (IRBs) of each institution.

Epidemiological data collection

After obtaining informed consent, participants were interviewed by one trained interviewer who was blinded to each subject's disease status. Participants completed questionnaires structured to elicit information related to sociodemographic characteristics, individual medical history, and family history of cancer. Sociodemographic characteristics included education, occupation, cigarette smoking, alcohol consumption, and physical activity, with a detailed time frame of exposures. Pathology and laboratory data were collected, recorded, and entered into the epidemiological database. Medical charts and pathology reports were examined to ensure that control subjects had no history of cancer.

Blood sample collection

Twenty mL of peripheral venous blood was obtained after a period of fasting from each enrolled subject before the initiation of treatment. Samples were transported to the laboratory without revealing the disease status prior to analysis of antioxidant micronutrients. All blood samples were centrifuged within 1 h following collection. Following separation of plasma, samples were stored at –80°C until assayed.

High-pressure liquid chromatography analysis of serum antioxidants

The concentrations of serum carotenoids, retinol, and tocopherols were measured using high-performance liquid chromatography (HPLC) (9). Serum samples from study subjects were randomized and analyzed together in the same batch under subdued light. β-Carotene, lycopene, zeaxanthin, lutein, α-tocopherol, and γ-tocopherol were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All solvents (methanol, ethanol, chloroform, n-hexane, acetonitrile, and tetrahydrofuran) were purchased from J.T. Baker (Phillipsburg, NJ, USA) and were filtered through a 0.2-μm membrane filter (Waters, Millipore, MA, USA) before use. Serum carotenoids, retinol, and tocopherols were extracted using $CHCl_3$-CH_3OH (2:1, v/v). Retinol acetate and tocopherol acetate were added as internal standards for the analysis of retinol, carotenoids, and tocopherols. All sample processing was performed under red light.

To separate the four carotenoids, retinol, and two tocopherols simultaneously, a gradient mobile phase was used. The detector was set at 292 nm for tocopherols, 340 nm for retinol, and at 450 nm for carotenoids. Individual carotenoids, retinol, and tocopherols were quantitated using peak areas calibrated against the standards. Loss due to handling loss was corrected by measuring the recovery of the internal standards. Because zeaxanthin and lutein isomers cannot be separated using the method we employed, they were eluted together and are hereafter referred to as zeaxanthin plus lutein. The mean coefficient of variation was 8% for retinol acetate and tocopherol acetate. Variation between duplicate serum samples was $<1\%$. The coefficients of variation were $<8\%$ for β-carotene, lycopene, and zeaxanthin plus lutein. The relative standard deviation (SD) for pooled controls ranged from 4% to 10%. The concentration of the standard solution was determined spectrophotometrically using the extinction coefficient E at 275 nm (E_10–3 = 14,200). Results were calculated using the ratio of the peak area of the compound over the peak area of the internal standard.

Statistical analysis

Fisher's exact test was used to compare the frequency distributions of categorical demographic variables among the four groups. Mean values and SDs were calculated as continuous demographic variables, with the mean differences tested using ANOVA and Duncan's multiple comparison. The geometric mean (95% confidence interval) was calculated for the serum concentrations of the carotenoids, retinol, and the tocopherols. ANCOVA was used to compare the mean val-

ues of the serum concentrations of the carotenoids, retinol, and the tocopherols following log transformation (SAS, SAS Institute, Inc, Cary, NC, USA) (10), with adjustments for potential confounders such as age, menopause, parity, oral contraceptive use, smoking status, alcohol consumption, and HPV infection status (age as a continuous variable, parity as a trichotomous variable, and the others as dichotomous variables).

Results

General characteristics of study subjects

The general characteristics of the study subjects are presented in Table 1. There were significant differences between controls and cases with respect to mean age and mean body mass index (BMI). The mean age for the controls was 46.8 years, while the mean ages for the CIN 1, CIN 2/3, and cervical cancer groups were 40.0, 40.2, and 51.5 years, respectively. The mean BMI for the controls was 22.8 kg/m^2, while the BMI values for the CIN 1, CIN 2/3, and cervical cancer groups were 22.3, 21.9, and 23.7 kg/m^2, respectively.

There were significant differences between controls and cases with respect to education level ($p<0.0001$), monthly household income ($p<0.0001$), and menopause status ($p<0.0001$). There were no significant differences in active smoking status among the four study groups, whereas the mean duration of exposure to passive smoke at their place of employment in CIN 2/3 group was significantly higher than that of the control group ($p=0.009$, $p<0.05$, respectively).

Table 1 General characteristics of study subjects.

Variables	Control	CIN 1	CIN 2/3	Cervical cancer	Total
Age, mean±SD, years	46.8±10.1	40.0±11.3[a]	40.2±10.1[a]	51.5±12.2[a]	
BMI, mean±SD, kg/m^2	22.8±2.8	22.3±3.1	21.9±3.1[a]	23.7±3.5[b]	
Education level, n/%					
≤Elementary	48/13	19/13	30/17	60/38	157
Middle school	54/14	12/8	21/12	27/17	114
High school	158/42	59/40	84/47	59/37	360
≥University	117/31	56/39	42/24	14/8	229
p-Value		0.181	0.161	<0.0001	
Monthly household income, n/%					
<1000 US $	46/13	19/13	22/13	43/31	130
1000–1999 US $	54/15	25/17	31/18	32/23	142
2000–2999 US $	62/17	32/22	45/26	25/18	164
3000–3999 US $	69/19	24/17	41/24	21/15	155
≥4000 US $	130/36	44/31	31/19	18/13	223
p-Value		0.553	0.0007	<0.0001	
Cigarette smoking, n/%					
Never	343/91	124/84	152/86	140/88	759
Current[d]	23/6	16/11	16/9	14/9	69
Former[e]	12/3	7/5	9/5	6/3	34
p-Value		0.105	0.225	0.495	
Passive smoking[f], n/%					
Mean±SD, min/week, home	455±654	379±590	451±958	372±532	
Mean±SD, min/week, workplace	457±996	636±1409	878±1666[c]	412±1116	
Alcohol consumption, n/%					
Mean±SD, years	15.9±7.5	12.1±7.6[a]	12.2±7.0[a]	16.7±7.8	
Ever use oral contraceptive, n/%					
No	320/85	116/79	144/81	129/81	709
Yes	58/15	31/21	33/19	30/19	152
p-Value		0.086	0.531	0.569	
Ever use multivitamins, n/%					
No	194/60	89/69	131/82	113/81	527
Yes	132/40	40/31	28/18	26/19	226
p-Value		0.060	<0.0001	<0.0001	
Menopause, n/%					
Yes	166/44	32/22	32/18	108/67	338
p-Value		<0.0001	<0.0001	<0.0001	
Number of childbirth, n/%					
1	44/13	11/11	20/14	15/10	90
2	192/58	66/63	90/65	75/51	423
≥3	93/28	27/26	29/21	58/39	207
p-Value		0.611	0.247	0.055	
Age at menarche, mean±SD, years	14.6±1.8	14.6±2.8	14.5±1.9	15.2±1.7[b]	
Age at 1st delivery, mean±SD, years	25.0±3.9	25.0±3.9	25.4±3.7	23.6±3.4[a]	

[a]$p<0.001$. [b]$p<0.01$. [c]$p<0.05$. [d]Those who had smoked any type of tobacco within the past 12 months. [e]Those who had stopped smoking at least 12 months previously. [f]Those who frequently stay in rooms where people smoke. Percentage values calculated only for subjects whose data were available. CIN, cervical intraepithelial neoplasia; BMI, body mass index; SD, standard deviation.

The mean duration of alcohol consumption, when compared with that of the controls (15.6 years), was significantly shorter in CIN 1 (12.1 years, $p<0.0001$) and CIN 2/3 (12.2 years, $p<0.0001$) patients. Also, there were significant differences between controls and cervical cancer cases in the use of multivitamins ($p<0.0001$), mean age at menarche ($p<0.01$) and first delivery ($p<0.001$).

Mean serum concentrations of antioxidant micronutrients

The mean serum concentrations of antioxidant micronutrients in study subjects are presented in Table 2. The mean concentrations of β-carotene and lycopene in CIN 1, CIN 2/3, and cervical cancer patients were significantly lower than those of controls. Mean zeaxanthin plus lutein concentrations were significantly lower for cervical cancer patients, but higher for CIN 1 patients compared with controls. The mean concentration of retinol was significantly higher for CIN 1 patients. Mean γ-tocopherol concentrations were ~25% of α-tocopherol concentrations in all study groups, and both tocopherols were significantly lower in CIN 2/3 and cervical cancer patients compared with controls.

Serum antioxidant micronutrients and risk of cervical carcinogenesis

Table 3 shows the dose-response relationship between serum antioxidant micronutrients and the odds ratio (OR) for CIN 1, CIN 2/3, and cervical cancer patients following adjustment for age, menopause, parity, oral contraceptive use, smoking status, alcohol consumption, and HPV infection status. Approximately 60% of the case subjects were HPV positive using the Hybrid Capture® 2 tests. We found negative trends in the OR for CIN 1, CIN 2/3, and cervical cancer associated with β-carotene, lycopene, and γ-tocopherol. There were negative trends in the OR for CIN 2/3 and cervical cancer with respect to α-tocopherol. In contrast, the results suggested that higher circulating levels of retinol were associated with a significant increase in the risk of CIN 1 and CIN 2/3. Higher circulating levels of zeaxanthin plus lutein were associated with a significant decrease in the risk of cervical cancer but showed positive trends for CIN 1, despite the fact that these results were statistically non-significant.

Discussion

Epidemiological studies show that HPV infection is a risk factor for the subsequent development of cervical cancer (11). Women with identified oncogenic types of genital HPV infections having advanced graded CIN are considered to have a high risk of developing cervical cancer (12). However, little is known about the factors or mechanisms that may influence the progression or spontaneous regression of CIN lesions. The role of diet and nutrition and risk of HPV persistence and cervical carcinogenesis was recently reviewed by Garcia-Closas et al. (13). They concluded that a likely protective effect against cervical neoplasia was afforded by folate, retinol, and vitamin E and possible for vegetables, vitamin C, vitamin B12, α-carotene, β-carotene, lycopene, zeaxanthin/lutein, and cryptoxanthin. In the current study, we report significantly decreased serum levels of antioxidant micronutrients, such as β-carotene, lycopene, zeaxanthin plus lutein, retinol, α-tocopherol, and γ-tocopherol in patients with CIN and cervical cancer.

Our data showed that cervical cancer was associated with older age, increased BMI, and lower socioeconomic status, as assessed by education and income. Research in other patient populations has documented an increased risk of cervical cancer among women of lower socioeconomic status (14, 15). In our study, women with less than an elementary school education and an income <$1000 per month were at increased risk of developing cervical cancer. These findings imply that interventions designed to prevent cervical cancer must ensure that all women of lower socioeconomic status have access to HPV vaccines, as well as access to education and other preventive services. Numerous studies have shown a positive association between oxidative stress measures and adiposity (16, 17). Also important, serum vitamin concentrations are progressively lower with increases in BMI, with the most obese having the lowest vitamin concentrations (18). Also, our data showed that increased BMI was associated with cervical cancer and that BMI was negatively correlat-

Table 2 Geometric mean levels of plasma micronutrients of study subjects.

	Control (n=378)	CIN 1 (n=147)	CIN 2/3 (n=177)	Cervical cancer (n=160)	Two-sided p for linear trend
Total carotenes, μmol/L	1.30	1.25	1.23	0.99	0.0001
β-Carotene, μmol/L	0.37	0.29	0.30	0.22	<0.0001
Lycopene, μmol/L	0.01	0.009	0.009	0.009	<0.0001
Zeaxanthin+lutein, μmol/L	0.73	0.89	0.85	0.70	0.0021
Retinol, μmol/L	2.17	2.49	2.23	2.12	<0.0001
Total tocopherols, μmol/L	3540	3357	2988	2960	<0.0001
α-Tocopherol, μmol/L	2818	2672	2333	2412	<0.0001
γ-Tocopherol, μmol/L	668	640	612	498	<0.0001

CIN, cervical intraepithelial neoplasia.

Table 3 Odds ratio of cervical neoplasia by quartile of serum antioxidant micronutrients.

Micronutrient/ quartile	Quartile range, ng/mL	No. of controls, %	CIN 1			CIN 2/3			Cervical cancer		
			No.	Crude OR, 95% CI	Adjusted OR[a], 95% CI	No.	Crude OR, 95% CI	Adjusted OR[a], 95% CI	No.	Crude OR, 95% CI	Adjusted OR[a], 95% CI
β-Carotene											
Low	1.74–15.3	94	65	1 (ref.)	1 (ref.)	74	1 (ref.)	1 (ref.)	104	1 (ref.)	1 (ref.)
2	15.3–21.6	95	47	0.72, 0.45–1.15	0.69, 0.41–1.14	47	0.63, 0.40–1.00	0.57, 0.34–0.94	29	0.28, 0.17–0.46	0.28, 0.17–0.48
3	21.6–27.4	94	16	0.25, 0.13–0.46	0.28, 0.15–0.54	32	0.43, 0.26–0.72	0.41, 0.24–0.72	16	0.15, 0.09–0.28	0.14, 0.07–0.26
High	27.4–60.1	95	19	0.29, 0.16–0.52	0.29, 0.16–0.55	24	0.32, 0.19–0.55	0.30, 0.16–0.54	11	0.11, 0.05–0.21	0.10, 0.05–0.21
p_{trend}				<0.0001	<0.0001		<0.0001	<0.0001		<0.0001	<0.0001
Lycopene											
Low	0.36–0.49	92	47	1 (ref.)	1 (ref.)	67	1 (ref.)	1 (ref.)	63	1 (ref.)	1 (ref.)
2	0.49–0.55	95	39	0.74, 0.45–1.22	0.70, 0.41–1.20	51	0.73, 0.46–1.15	0.74, 0.45–1.22	41	0.52, 0.32–0.83	0.55, 0.33–0.92
3	0.55–0.62	94	27	0.52, 0.30–0.89	0.52, 0.29–0.93	37	0.53, 0.33–0.87	0.56, 0.33–0.95	28	0.36, 0.21–0.60	0.34, 0.19–0.58
High	0.62–2.37	94	28	0.53, 0.31–0.92	0.51, 0.29–0.90	19	0.27, 0.15–0.49	0.27, 0.15–0.50	12	0.15, 0.08–0.30	0.16, 0.08–0.33
p_{trend}				0.040	0.032		<0.0001	<0.0001		<0.0001	<0.0001
Zeaxanthin + lutein											
Low	6.97–39.0	94	42	1 (ref.)	1 (ref.)	48	1 (ref.)	1 (ref.)	80	1 (ref.)	1 (ref.)
2	39.0–49.3	95	20	0.47, 0.26–0.86	0.50, 0.26–0.96	36	0.74, 0.44–1.25	0.70, 0.40–1.22	30	0.37, 0.22–0.62	0.38, 0.22–0.65
3	49.3–66.7	94	46	1.10, 0.66–1.82	1.56, 0.89–2.75	46	0.96, 0.58–1.57	1.12, 0.66–1.91	21	0.26, 0.15–0.46	0.23, 0.13–0.42
High	66.7–224	95	39	0.92, 0.55–1.55	1.40, 0.78–2.51	47	0.97, 0.59–1.59	1.23, 0.72–2.10	29	0.36, 0.22–0.60	0.33, 0.19–0.57
p_{trend}				0.619	0.053		0.809	0.216		<0.0001	<0.0001
Retinol											
Low	23.8–51.7	94	19	1 (ref.)	1 (ref.)	37	1 (ref.)	1 (ref.)	50	1 (ref.)	1 (ref.)
2	51.7–63.0	95	23	1.20, 0.61–2.34	1.33, 0.65–2.74	34	0.91, 0.53–1.57	0.97, 0.54–1.74	27	0.53, 0.31–0.93	0.47, 0.26–0.85
3	63.0–75.0	94	45	2.37, 1.29–4.35	2.85, 1.47–5.48	47	1.27, 0.76–2.13	1.60, 0.92–2.81	40	0.80, 0.48–1.33	0.69, 0.40–1.20
High	75.0–146	95	60	3.13, 1.73–5.63	4.56, 2.37–8.76	59	1.58, 0.96–2.60	2.25, 1.30–3.89	43	0.85, 0.52–1.40	0.74, 0.43–1.26
p_{trend}				<0.0001	<0.0001		0.031	0.0009		0.805	0.458
α-Tocopherol											
Low	214–986	94	50	1 (ref.)	1 (ref.)	69	1 (ref.)	1 (ref.)	67	1 (ref.)	1 (ref.)
2	986–1210	95	39	0.77, 0.47–1.28	0.95, 0.55–1.64	61	0.88, 0.56–1.37	0.90, 0.56–1.46	49	0.72, 0.45–1.15	0.52, 0.31–0.87
3	1210–1475	94	27	0.54, 0.31–0.94	0.74, 0.41–1.36	32	0.46, 0.28–0.77	0.59, 0.34–1.02	26	0.39, 0.23–0.66	0.24, 0.13–0.43
High	1475–4063	95	31	0.61, 0.36–1.04	0.94, 0.52–1.69	15	0.22, 0.12–0.40	0.30, 0.16–0.59	18	0.27, 0.15–0.48	0.19, 0.10–0.37
p_{trend}				0.043	0.712		<0.0001	0.0002		<0.0001	<0.0001
γ-Tocopherol											
Low	46.0–224	94	45	1 (ref.)	1 (ref.)	55	1 (ref.)	1 (ref.)	85	1 (ref.)	1 (ref.)
2	224–287	95	41	0.90, 0.54–1.50	0.88, 0.51–1.51	52	0.94, 0.58–1.50	0.81, 0.48–1.35	37	0.43, 0.27–0.70	0.42, 0.25–0.71
3	287–378	94	35	0.78, 0.46–1.32	0.69, 0.39–1.23	44	0.80, 0.49–1.30	0.66, 0.39–1.13	24	0.28, 0.17–0.48	0.30, 0.17–0.53
High	378–964	95	26	0.57, 0.33–1.00	0.54, 0.30–1.00	26	0.47, 0.27–0.81	0.41, 0.23–0.74	14	0.16, 0.09–0.31	0.18, 0.09–0.35
p_{trend}				0.041	0.037		0.004	0.002		<0.0001	<0.0001

[a]Adjusted for age, menopause, parity, oral contraceptive, smoking status, alcohol consumption, and HPV infection status. CIN, cervical intraepithelial neoplasia; OR, odds ratio; CI, confidence interval; ref., reference (the lowest quartile was used as the reference).

ed with serum concentrations of antioxidant micronutrients.

We found that controls had a tendency to use or had previously used multivitamins compared with patients with cervical cancer (40% vs. 19%) ($p < 0.0001$). Although many studies, including the Supplementation in Vitamins and Mineral Antioxidants (SU.VI.MAX) study, have failed to demonstrate the role of antioxidant supplementation in the prevention of cancer (19–21), it is possible that the use of multivitamins may influence the increased levels of serum antioxidants in controls. Unlike most previous research in this field, our study showed that current cigarette smoking was not related to the risk of cervical cancer, while exposure to passive smoke seemed to be related to the risk of CIN 2/3. Although a consistent association between cigarette smoking and cervical cancer has been noted in numerous studies (22, 23), the relationship between smoking and cervical cancer is difficult to prove. This is because of the strong confounding effect of sexual behavior, such as number of sexual partners and exposure to other sexually transmitted diseases. Because the current study did not capture this type of information about potential confounding variables, our findings could have overestimated or underestimated the risk of cigarette smoking on development of cervical neoplasia.

Several studies have suggested a protective effect of β-carotene against cervical dysplasia, but none of the findings were statistically significant. In a randomized clinical trial investigating the association of β-carotene with early preinvasive lesions, oral administration of β-carotene had no effect on atypical squamous cells of undetermined significance (ASCUS)-CIN1 regression rates during 2 years of follow-up (24). Results from a case-control study by Nagata et al. showed an inverse association between serum lycopene concentrations and cervical dysplasia (25). The results of our study support the hypothesis that increased blood concentrations of several carotenoids, especially β-carotene and lycopene, may decrease the risk of cervical cancer. The risk of cervical cancer was decreased the greatest in women with high circulating concentrations of β-carotene, and this relationship was most pronounced in the subgroups of older women and those who consumed alcohol (data not shown). We also found that increased serum concentrations of several carotenes were associated with a decreased risk of CIN. Zeaxanthin plus lutein showed positive trends in the OR for CIN 1 and CIN 2/3, but there was no statistical significance.

With respect to retinol, several observational studies have shown a protective effect of serum retinol against cervical neoplasia (25, 26). Clinical trials that have evaluated the effects of topically applied retinoic acid showed an increase in the regression rates of CIN 2 but not CIN 3, lending support to its efficacy in patients with early lesions. Our data are inconsistent with the majority of other studies concerning the association of serum retinol and risk of cervical dysplasia. We found that high serum concentrations of retinol were associated with an increased risk of CIN 1 and CIN 2/3. However, serum retinol showed a negative, but non-significant, trend in the OR for cervical cancer. We suspect that the lack of association between retinol and cervical dysplasia found in our study may be due to the small differences in serum concentrations of retinol observed between controls and patients with cervical cancer cases, especially when compared differences seen with the other antioxidant micronutrients.

Few studies have examined the relationship between serum tocopherols and risk of dysplasia. Goodman et al. measured serum micronutrient concentrations and found a significant inverse association between α-tocopherol and risk of cervical dysplasia after adjustment for HPV and other confounders (27). In our studies, high serum concentrations of α-tocopherol and γ-tocopherol were associated with reduced risk of CIN and cervical cancer, although the relationship between the serum concentrations of α-tocopherol and risk of CIN 1 revealed a marginal inverse correlation. Unfortunately, we could not compare the corrected tocopherols for serum lipids because only partial data on blood lipids were available.

There are possible biological mechanisms by which micronutrients may protect against the progression of cervical carcinogenesis. Vitamin C, vitamin E, carotenoids, and other dietary constituents could act as efficient scavengers of free radicals and oxidants. These substances, produced during normal metabolism, inflammatory processes, and also present in high amounts in tobacco smoke, could lead to extensive damage of DNA, proteins, and lipids if not neutralized by antioxidant molecules (28). It has been hypothesized that vitamin C, α-tocopherol, and other antioxidants may reduce the risk of cervical carcinogenesis by blocking nitrosamine formation from tobacco smoke (29). Dietary components that possess antioxidant properties may protect the immune system from oxidative damage and enhance immune responsiveness (30). Immune cells are particularly vulnerable to oxidative stress. Thus, antioxidant levels in these cells play an important role in maintaining a reduced environment and preserving cellular function.

Our study has potential limitations that must be considered in the interpretation of our results. First, recall bias is always a potential problem in case-control research. To address some of the potential problems associated with this type of research, we used a structured interview and a standard protocol for cases and controls. Second, we collected and measured the serum concentrations of antioxidant micronutrients at the time of diagnosis. It is possible that micronutrients may not have the same protective effects following the genesis of cervical dysplasia. As sera was not obtained from cases prior to the diagnosis of cervical dysplasia, we cannot exclude the possibility that the observed associations may be a consequence of disease and not a reflection of the

etiology the disease. In addition, a single determination of antioxidant micronutrients at a single point in time may be a poor indication of long-term dietary exposure (31). Nevertheless, findings from cohort studies of blood concentrations of antioxidant micronutrients are generally consistent with the findings from dietary epidemiological studies. Kardinaal et al. reported correlation values close to 0.90 for both β-carotene and α-tocopherol from repeat blood draws from the same individuals collected 4 months apart (32). Therefore, analysis of these antioxidant micronutrients from a single blood collection is likely to classify individuals appropriately in epidemiological studies. A third potential limitation is that HPV data were limited to the collection of a single specimen at study entry. Additional sampling or HPV detection by polymerase chain reaction (PCR) dot-blot hybridization would likely have resulted in a higher proportion of patients showing HPV infection (33).

Despite these potential limitations, we found inverse associations between serum antioxidant micronutrients, especially β-carotene, lycopene, α-tocopherol, and γ-tocopherol, and the development of cervical neoplasia after adjusting for HPV infection, cigarette smoking, alcohol consumption, and other confounders. These results suggest that antioxidant micronutrients may play a role in the prevention of cervical carcinogenesis.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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